Continental J. Biological Sciences 3: 63 - 74, 2010 ISSN: 2141 - 4122

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# FERMENTATION OF SORGHUM USING YEAST (*Saccharomyces cerevisiae*) AS A STARTER CULTURE FOR BURUKUTU PRODUCTION

[1]Mbajiuka Chinedu S , [2]Omeh Yusuf S and [3]Ezeja Maxwell.I.
[1]Department of Microbiology, [2]Department of Biochemistry,College of Natural and Applied Sciences, and [3]Department of Veterinary Physiology, Pharmacology and Biochemistry Micheal Okpara University of Agriculture,Umudike,Abia State,Nigeria.

ABSTRACT
The traditional method of burukutu production involves malting, mashing, addition of an adjunct, fermentation of sorghum using an old brew as a starter culture for 48 h pasteurization by boiling and maturation. The use of *Saccharomyces cerevisiae* as a starter culture in burukutu production was compared with the traditional method of brewing burukutu. The microorganisms associated with the traditional method include *Staphylococcus* species, *Streptococcus* species, *Enterobacter* species, *Candida* species, *Aspergillus* species *and Saccharomyces* species. The titratable acidity was gradually increasing from 0.20 to 0.36M for old brew and 0.21 to 0.38M for yeast only. The pH decrease as fermentation proceeds from 5.86 to 3.67f for old brew and from 5.91 to 3.57 for yeast only at room temperature. The percentage of reducing sugar and specific gravity decrease with increase in fermentation period. The fermentation rate was higher for the case of inoculating with *Saccharomyces cerevisiae* compared with inoculating with old brew. The percentage alcohols of both samples were gradually increasing as the fermentation proceeds but higher in the sample inoculated with *Saccharomyces cerevisiae* after 48 h with 0.14%. The effect of nutritional contents, shelf life and other qualities of burukutu beer needs to be investigated.

KEYWORDS:-Traditional methods, Fermentation, *Saccharomyces cerevisiae*, starter culture, burukutu.

## INTRODUCTION

Cereals are more widely utilized as food in African countries than in the developed world. In fact cereals account for as much as 77% of total calorie consumption in African countries (Alais and Linden,1999;Norman, *et al*, 1999). A majority of traditional cereal-based food as shown in table 1 consumed in Africa and mainly processed by natural fermentation. Fermented cereals are important as dietary staples for adults in Africa. Major cereals grown in Africa include sorghum, rice, maize and millet (Norman, *et al*, 1999).

Sorghum is one of the cereals cultivated in the tropical region of Africa and is about the largest cultivated crop in the northern Guinea savanna areas of Nigeria (Kolawole, *et al*, 2007). Sorghum is a large variable genus with many cultivars. It constitutes a major source of energy and it serves as a staple food of many of the world's poorest and least privileged people (Hamad *et al*, 1993 and Michodjehoun-Mestres, *et al*, 2005).

According to Ahmed *et al*.,1996,sorghum products have poor nutritional value due to their deficiency in lysine, threonine and tryptophan and presence of anti-nutritional factors such as tannins and phytates that interact with proteins, vitamins and minerals, thus restricting their bio-availability. The above factors contribute to anemia and other nutritional diseases in developing countries where the consumption of sorghum products is high(Hassan and El Tinay,1995). However, various techniques have been investigated to improve the protein digestibility and mineral availability of sorghum by reducing its tannin and phytate content. These include malting, fermentation and cooking (Abd Elmoneom *et al*.,*2005* and Okafor,1981.) Fermentation is the process of anaerobic oxidation of carbohydrates to produce intermediate substrates (organic acid, ethanol etc) with the release of carbon dioxide (Prescott *et al*, 2005). The advantages of

fermentation can not be over emphasized. It is one of the important techniques employed to extend the shelf life of raw food materials while in the technically advanced countries, it is used more to develop and add flavour to variety of diet (Achi, 2005; Isabel *et al*, 2005). Fermentation is widely used traditionally for processing sorghum into fermented products. Their low pH confers the advantage of microbiological safety (Tomkins *et al.*, 1988). Sorghum based foods includes burukutu, pito, bogobe, kisra, injera etc. They are mainly fermented; some are non-alcoholic while others are alcoholic beverages(Booney,2005;Sulma *et al.*,1994 and Okafor,1981).

Burukutu is a popular indigenous alcoholic beverage of a vinegar-like flavour, consumed in the Northern Guinea savanna region of Nigeria,Republic of Benin and in Ghana (Kolawole *et al*, 2007 and Norman *et al*, 1999). It is mainly produced from the grains of guinea corn (*Sorghum vulgare and Sorghum bicolor)*. The traditional process of preparing burukutu involves steeping sorghum grains in water overnight, malting, mashing, fermentation and maturation as described by Norman *et al*, 1999 and Achi, (2005). It is a batch process carried out on a small scale. Burukutu as an indigenous beer brewed at the cottage level in some parts of West Africa has basic characteristics that include a sour taste due to the presence of lactic acid, a pH of 3.3 to 3.5 and opaque colour because of suspended solids and yeast. It contains vitamins, iron, manganese, magnesium, phosphorus and calcium and also contains about 26.7g of starch and 5.9g of protein per liter (Jogo *et al*.,2002;Egemba and Etuk, 2007). However, some of the endogenous sorghum microorganisms are pathogenic or may produce toxic substances, such as mycotoxins. (Isabel *et al*, 2005). But pasteurization of freshly brewed burukutu sample at $60^0$C for 30 min delayed its spoilage for two weeks (Alais and Linden ., 1999).

Considering the importance of traditional fermentation of cereal-based beverage and the need of using a single microbe as starter culture, it became necessary to investigate the possibility of using a starter culture (such as yeast *Saccharomyces cerevisiae)* in burukutu production and comparing it with burukutu produced by the normal traditional method (using old brew as starter culture).

TABLE 1: FERMENTED CEREALS BASED FOODS

| Cereal | Food | Class | Location |
|---|---|---|---|
| Sorghum | Burukutu | Alcoholic beverage | Northern part of Nigeria, Ghana |
| | Pito | Alcoholic beverage | Mid-western part of Nigeria, |
| | Obiolor | Non-alcoholic beverage | Igala in Nigeria |
| | Ogi | gruel | Nigeria |
| | Bogobe | Sorghum porridge | Botswana |
| | Kisra | Sorghum bread | Sudan |
| | Injera | Sorghum bread | Ethiopia |
| | Merrisa | Alcoholic drink | Sudan |
| Maize | Ogi | Gruel | Nigeria |
| | Kenkey | Solid/dough | |
| Millet | Kunuzaki | Non-alcoholic drink | Northern part of Nigeria |
| Wheat | Bouza | Alcoholic beverage Hamma | Egypt and some Arabian countries |
| | Kishk | | |
| Barley | Beer | Alcoholic drink | Nigeria |

Adapted from Norman *et al*,1999; Achi, 2005 and Achi, 1990.

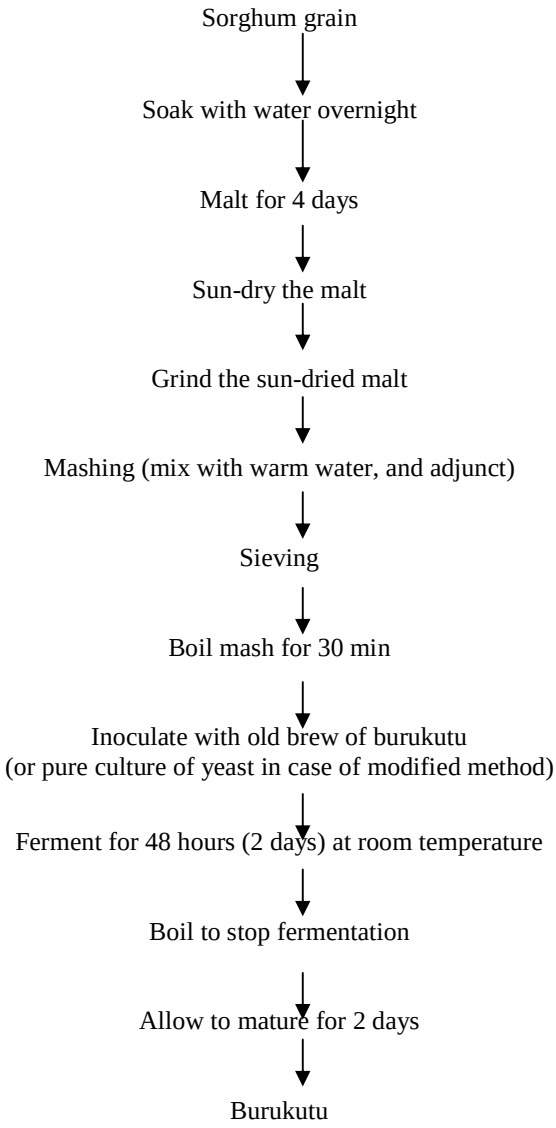
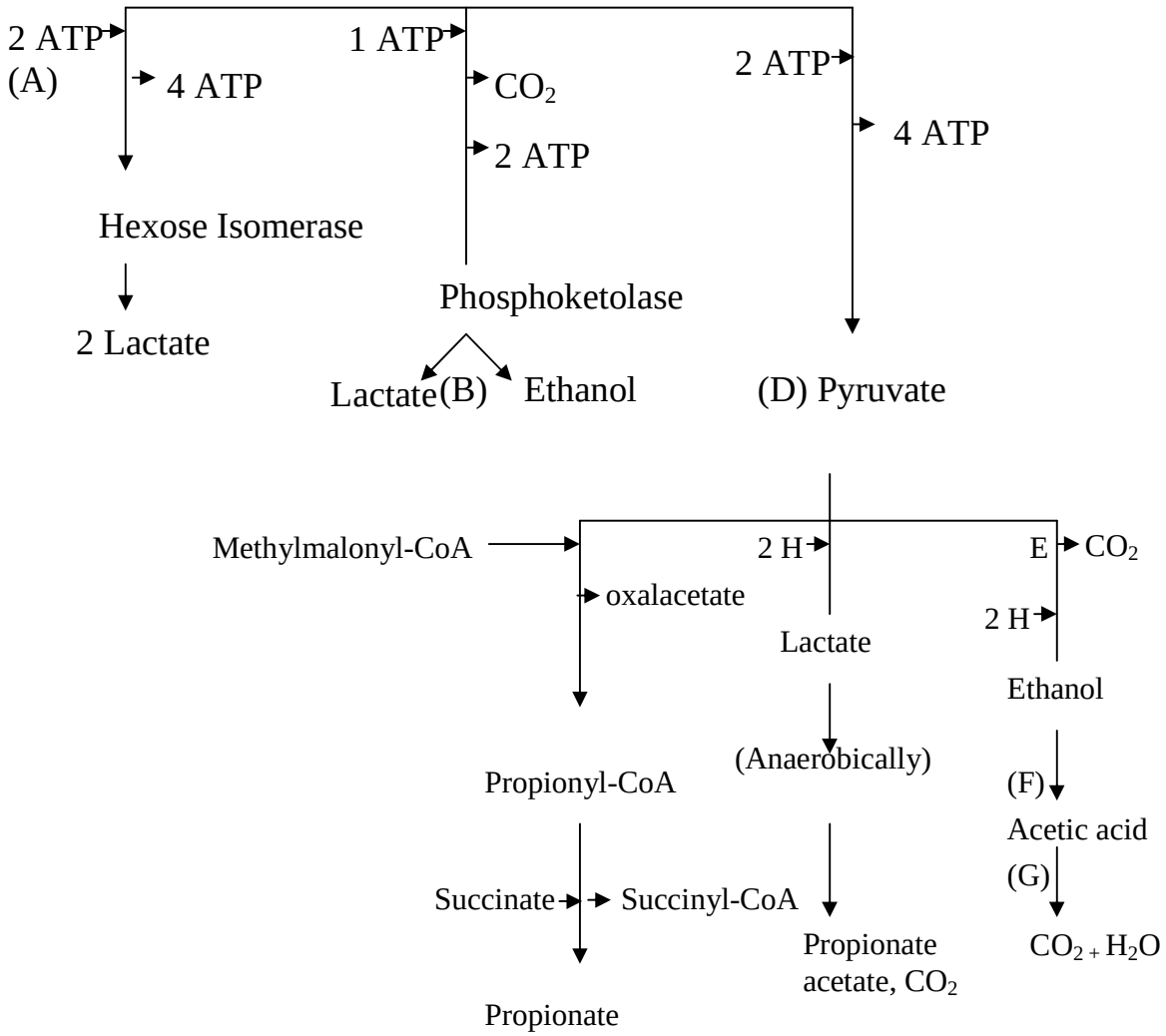



Figure 1: Flow Sheet For The Traditional and Modified Method of Burukutu Production From Sorghum Grain.




A:*Homofermentative lactics*
B:*Heterofermentative lactics*
C and D:*Propionibacterium*
E:*Saccharomyces spp*
F:*Acetobacter spp*
G:*Acetobacter*"overoxidizer"

Figure 2: Generalized pathways for the production of some fermentation products from glucose by various organisms represented by letters A to G.
Source:Alais and Linden,1999.

MATERIALS AND METHODS
MATERIALS
SORGHUM
The red variety of sorghum *(Sorghum vulgare)* used for this work was purchased from Umuahia main market in Abia State, Nigeria.

OLD BREW
The old brew of burukutu used for inoculation in line with traditional method of burukutu production in this study was obtained from the open market in Gariki, Okigwe, Imo state, Nigeria.

YEAST
The yeast strain, *Saccharomyces cerevisiae* used in this work was obtained from the central laboratory at the National Root Crops Research Institute Umudike in Abia State, Nigeria.

METHODS
MALTING
The dehulled and cleaned sorghum grains were steeped overnight in a plastic container with tap water at room temperature (28-30$^0$C) without changing the water. The soaked grains were washed and drained. They were uniformly spread on a wet sack cloth, then covered with banana leaves to reduce dehydration. The grains were kept wet by frequent spraying with tap water every morning, and turning over at intervals. Germination was done at room temperature (28-30$^0$C) for four days (Michojehoun-Mestres *et al*, 2005 and Achi, 2005).

SUN DRYING
The germinated or malted sorghum grains were sun-dried in accordance with traditional practice (Kolawole *et al*, 2007 and Mohammed *et al.*,1999).

MILLING
The sun-dried malted sorghum grain was milled into flour and passed through a 0.5mm sieve, using a community plate disc mill. The flour was stored in a polyethylene bag at an ambient temperature prior to analysis according to Kolawole *et al*, 2007.

MASHING
The mashing of the sorghum malt was carried out by a method as described by Egemba and Etuk, (2007) and Achi, (2005). 3.0kg of sorghum malt was weighed with a weighing balance and mixed with adjunct (a farinaceous fermented cassava product) and warm water (45$^0$C) in the ratio of one part garri to two part malt and six part water (1:2:6). The mixture was stirred and allowed to settle for 30min. when settled, 2L of the clear enzymatic supernatant was decanted and the remaining mash was gradually brought to boil at 100$^0$C for 30min. The mash was allowed to cool at 60$^0$C and the clear enzymatic supernatant was added and then kept for 12 h. The mixture was filtered through a sieve mash and rinsed with 30ml of water (45$^0$C) to extract the remaining enzymes from the grist. The part of the wort was further boiled for some minutes and allowed to cool for inoculating with pure culture of yeast.

PREPARATION OF STARTER CULTURE OF YEAST FOR INOCULCATION
One gram of dry *Saccharomyces cerevisiae* weighed with a weighing balance was added to 100ml of the sorghum wort with three tea spoons of glucose and left for six hours to activate the yeast strain. The activated yeast was inoculated into 400ml of sorghum wort for fermentation (Egemba and Etuk, 2007).

FERMENTATION OF SORGHUM WORT
The fermentation was carried out in a 2L fermentation bucket constructed with plastic tap for 48 hours at room temperature (Nout,1981and Sukki *et al*.,1994).

The natural fermentation was carried out by inoculating 100ml of old brew of burukutu into 400ml of the sorghum wort in line with the traditional method of brewing burukutu (Egemba and Etuk, 2007).

MEASUREMENT OF PARAMETERS
pH
The pH of the samples was determined using pH meter (Hanna pH 211) at interval of 12 h(Richard *et al*.,1999).

TEMPERATURE
The temperature of the samples was determined using pH meter (Hanna pH 211) with temperature electrode.

TITRATABLE ACIDITY
The titratable acidity was determined by titrating 2ml of samples with 0.1M NaOH to the phenolphthalein end point. The titratable acidity was expressed as the volume of NaOH solution required to neutralize the free acid contain in the sample. This method was described by Ogbadu *et al*, 1997.

FERMENTABLE SUGARS
The changes in fermentable sugars were determined using brix meter (Handheld model 1305) at 12h interval according to Onwuka, 2005.

SPECIFIC GRAVITY
The specific gravity of the samples was determined using brix meter (Handheld Model 1305) with specific gravity readings chart as also described by Bhrise *et al*.,(1988).

DETERMINATION OF ALCOHOLIC CONTENT
To determine the percentage of alcohol in the beverage, the specific gravity of the both samples before fermentation starts were compared with specific gravity after fermentation. Following the equation of converting glucose to alcohol by yeast, each glucose molecule is converted into two molecules of ethyl alcohol and two molecules of carbon dioxide.

$$C_6H_{12}O_6 \rightarrow 2(CH_3CH_2OH) + 2(CO_2).$$

Checking the molecular weight of the molecules, ethyl alcohol is equal to 46.0688 and carbon dioxide is 44.0089.

From the equation above, each carbon dioxide molecule that leaves the fermentation vessel, one ethyl alcohol molecule must be formed inside the vessel. From the molecular weight, each 44.0098 grams of $CO_2$ that leaves the vessel, 46.0688 grams of ethyl alcohol are formed. In other hand, for each gram of $CO_2$ that bubbles off, about 1.05 grams of ethyl alcohol are produced. Therefore, comparing the specific gravity of the beverage, the final specific gravity is subtracted from the first to give the molecular weight of $CO_2$ that left the vessel. Then multiply by 1.05 to get the weight of the alcohol per litre of the container. To determine the percentage of alcohol by mass, divide the mass of the alcohol with the specific gravity of the solution after fermentation. Hence this fomular:

$$\frac{\text{Mass of alcohol}}{\text{Final specific gravity}} \text{ X } 100$$

However, since the percentage of alcohol by mass is higher than the percentage of alcohol by volume because an equal mass of alcohol occupies more volume than water would. To convert from percent alcohol by mass to percent alcohol by volume, the percent alcohol by mass will be divided by density of alcohol which 0.79.

MICROBIAL ANALYSIS OF THE NATURAL FERMENTATION SAMPLE
Serial dilution of the natural sample ($10^{-3}$) was inoculated into a nutrient agar prepared with 0.2ml of antifungal (fluconazole) for bacteria isolation and sabouraud dextrose agar for fungi isolation for 24 h and 48 h respectively. Bacterial identification was carried out using gram staining reaction and Fungi identification was carried out by macroscopic and microscopic examination. The media were prepared according to the manufacture's instruction.The method is according to Glover *et al*.,(2005).

RESULTS

MALTING

The sorghum grains used in this project work were dehulled washed and steeped overnight in tap water. They were spread out on a sac bag and kept for germination. The germination of the sorghum grains commenced after 12 h with about 50% of them germinating and the germination increased to about 99.5% after 24 h. At the end of the malting period which was four (4) days plumules attended to about 1.5cm to 2cm in height as shown in plate 1. Besides, there was a scanty growth of moulds on the malting sorghum grains.

GROUND SUN-DRIED SORGHUM MALT

After the malting process, the germinated sorghum grains were sun-dried and ground into a fine powder. The ground malt releases a pleasant aroma and tasted sugary.

FERMENTATION

After grinding of sorghum malt, the powder mixed with an adjunct (garri) was mashed with addition of water to get a possible extract or wort which was subjected for fermentation by inoculating with a starter culture as shown plate 2. After 12 h, there was a presence of bubbles which first started in the sample inoculated with *Saccharomyces cerevisiae*. As the fermentation progress, an alcoholic flavour was observed from each of the samples. However, the following parameters were tasted at 12 h interval.

DETERMINATION OF pH VALUES

As the fermentation proceed. The pH of both inoculated fermenting samples decreases with time as shown in Figure 3.

TEMPERATURE DETERMINATION

The temperature of the fermenting medium was fluctuating within room temperature which ranges from $28.7^0$C to $30^0$C with increase in fermentation period as presented in Table 3.

TITRATABLE ACIDITY

The titration of 0.1M NaoH in 2ml of the samples at phenolphthalein end point shows a gradual increase in the acid content of the fermenting medium with time. The concentration of the acid as shown in Figure 4 was calculated.

REDUCING SUGARS

During the fermentation process, the levels of fermentable sugars were tasted using refractometer (Handheld model 1305) which was indicated by the appearance of blue color within the range. The percentage of sugar level was observed to be decreasing as the fermentation period progresses as shown in Figure 5.

SPECIFIC GRAVITY

Specific gravity is a measure of the density of a liquid relative to water. It was decreasing as the fermentation period increases in both samples as illustrated in Figure 6.

ALCOHOLIC CONTENT

The alcoholic content determined by comparing the specific gravity of the samples before and after fermentation shows that percentage alcoholic content of the samples was increasing with fermentation time but the sample inoculated with *Saccharomyces cerevisiae* which is 6.7% by volume was higher than that of sample inoculated with old brew which is 6.57% by volume after fermentation. Therefore the percent alcohol differs by 0.14%. This is illustrated in Figure 7.

IDENTIFICATION OF ISOLATES

The serial dilutions of $10^{-3}$ of the natural sample cultured on nutrient agar and sabouraud dextrose agar to identify bacterial and fungal isolates under aerobic conditions shows the list of microorganisms represented in Table 4 and 5 respectively.

TABLE 3: CHANGES IN TEMPERATURES OF THE FERMENTING SAMPLES WITH TIME.

| FERMENTATION TIME (h) | TEMPERATURE ($^0$C) | |
|---|---|---|
| | O.B | S.C |
| 0 | 28.7 | 28.8 |
| 12 | 28.9 | 29.7 |
| 24 | 29.6 | 29.8 |
| 36 | 29.8 | 30.0 |
| 48. | 30.0 | 30.0 |

Note: S.C = *Saccharomyces cerevisiae,* O.B = Old brew of burukutu

TABLE 4: SUSPECTED BACTERIA ISOLATES

| S/N | BACTERIA | GRAM REACTION |
|---|---|---|
| 1 | *Staphylococcus* species | Gram positive cocci in clusters |
| 2 | *Streptococcus* species | Gram positive cocci in chains |
| 3 | *Enterobacter* species | Gram negative rod |

TABLE 5: SUSPECTED FUNGI ISOLATES

| S/N | FUNGI | MACROSCOPIC IDENTIFICATION | MICROSCOPIC IDENTIFICATION |
|---|---|---|---|
| 1 | *Candida* species | White creamy, slightly raised | Cells are round to ellipsoid |
| 2 | *Saccharomyces* species | Brown creamy, round colonies | Cells are large, globose and also budding |
| 3 | *Aspergillus* species | Back colonies | Septate hyphae with V-shaped branching |

DISCUSSION

Sorghum grains are an important fermentable cereal used to produce indigenous alcoholic, non-alcoholic beverages, baked products and animal feeds.

Burukutu is one of the indigenous alcoholic beverages produced by the fermentation of malted sorghum grains within two (2) days. The process involves malting, sun-drying, grinding, mashing, fermentation; pasteurization and maturation as shown in Figure 1.

During malting of sorghum grains, starch is hydrolyzed into fermentable sugars mainly by amylolytic organisms capable of hydrolysing starchy constituents (Michodjehoun-Mestres *et al*, 2005 and Achi, 1990). At the end of malting which is four (4) days, the plumule attends to about 1.5 to 2cm in height as similarly observed by Achi, (1990). The scanty growth of moulds observed on the malting grains may be contaminants from the banana leaf used to cover the sorghum grains to prevent dehydration (Norman *et al*, 1999).

The release of pleasant aroma and sugary taste by the sun-dried grinded malt shows the effect of malting to improve flavour and conversion of starch to fermentable sugars.

In the process of fermentation, the appearance of bubbles and release of an alcoholic flavour after 12 h signifies the commencement of fermentation with release of $CO_2$ and formation of alcohol.

The isolated bacteria such as *Staphylococcus* species, *Enterobacter* species, *Streptococcus* species and Fungi such as *Aspergillus* species, *Candida* species *and Saccharomyces* species as shown in Table 4 and 5 were suspected as those that directly or indirectly participate in the fermentation with natural inoculum of old brew as starter culture. This was in line with Kolawole *et al*, (2007). According to Mohammed *et al*.,(1999), *Saccharomyces cerevisiae* and other yeasts are responsible for the alcoholic fermentation and also contribute to the flavour and acceptability of the product in combination with *Streptococcus lactis*, *Candida mycoderma* and *Lactobacillus* species.

However, the increase in titratable acidity leads to decrease in pH as fermentation time increases. But the sample inoculated with commercial yeasts which is *Saccharomyces cerevisiae* was faster than the natural one which was also confirmed with the work of Egemba and Etuk, (2007). This may suggest that the use of only *Saccharomyces cerevisiae* in sorghum fermentation to produce burukutu will encourages greater capability of producing acid than those of combined effort of those strains of organisms involved in natural fermentation. In addition, the increase in acidity with decrease in pH as fermentation proceed may eliminate or discourage the growth of most spoilage and pathogenic microorganisms that can not withstand such condition hence making the alcoholic beverage safer for consumption and also helps to increase the shelf life in combination with pasteurization by boiling.

In case of reducing sugars, the percentage of fermentable sugars decrease sharply with the inoculation of *Saccharomyces cerevisiae* than the old brew with increase in fermentation period as shown in figure five. This may signify the effect of higher utilization of fermentable sugars by *Saccharomyces cerevisiae* than other strains of microorganisms isolated from the natural fermentation process. Besides, the decrease in specific gravity of the samples shows that some of the sugars have been converted into alcohol which is less dense than water. A similar observation was made by Egemba and Etuk, (2007).

Furthermore, the increase in percentage of alcohol in both fermentation medium also suggests the continuous activity of the microorganisms in conversion of glucose to alcohol as fermentation proceeds. At the end of fermentation, the percentage of alcohol in the sample inoculated with *Saccharomyces cerevisiae* was higher with 0.14% than the one inoculated with old brew as shown in figure 7. A similar result was gotten by Kolawole *et al*, (2007) but difference in final percentage of alcohol in the natural method carried out in the laboratory may be as a result of differences in quantity of sorghum flour used and the variety.

After maturation, the increase in palatable taste and flavour which makes the burukutu more attractive for consumption shows the activity of microorganisms that dominate the beverage during the maturation as described by Achi, (2005) but more developed in the traditional method. The fermentation of Sorghum using only pure culture of palmwine yeasts (*Saccharomyces cerevisiae)* produced higher alcohol but not with similar aroma and flavour as in the traditional method. The aroma and flavour that makes burukutu attractive for consumption is present in the traditional method and is as a result of the activities of many other microorganisms present and this conforms with the work of Achi (1990).The implication of this result is that since we are not only interested in the alcoholic production but also in the characteristic aroma and flavour of burukutu that makes it attractive for consumption, the use of a single pure culture as starter culture in the production of burukutu using sorghum that could be acceptable is not feasible.

## CONCLUSION

This study reveals that fermentation rate were higher for the case of inoculation with commercial yeast *(Saccharomyces cerevisiae)*, compared to inoculating with old brew of burukutu as a traditional method of brewing burukutu hence suggesting that the use of a starter culture *Saccharomyces cerevisiae* (Yeast) would ensure better fermentation rate with higher production of alcohol in burukutu.

Our limitations included lack of funds to further our research and the unavailability of digital modern equipment for analysis which left us with the option of improvision sometimes.These limitations are characteristics of third world countries particularly African countries.

However, since our intentions are to improve and modernize the method of burukutu production using sorghum,the aroma , the nutritional value and other qualities of burukutu beer were taken for granted.Therefore,there is the need to investigate these and as well as their respective shell life.The roles played by many other microorganisms isolated from the back drop be investigated. We also suggest that the use of a combination of pure cultures of Lactic acid bacteria and *Saccharomyces cerevisiae* as a starter culture in burukutu production using sorghum be investigated to see if it could bring out good aroma.

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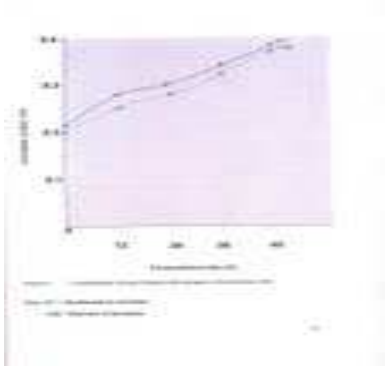

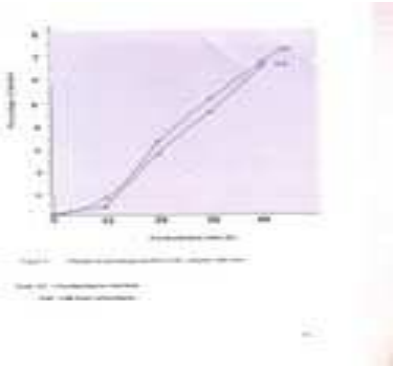

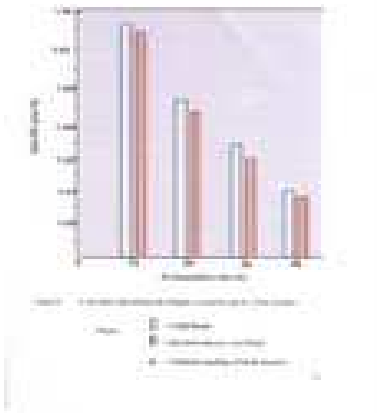

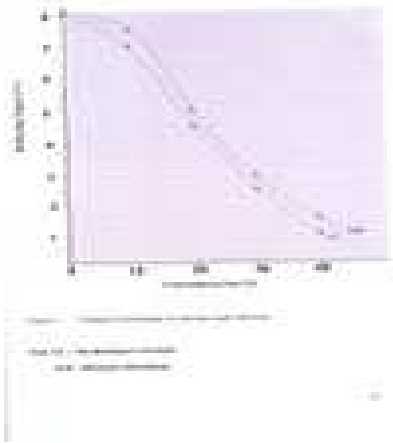

Received for Publication: 09/06/2010
Accepted for Publication: 21/07/2010

Corresponding author:
Omeh Yusuf S
Department of Biochemistry
Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria.
E-mail: nduk41@yahoo.com